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# Extremely small genomes in two unrelated dipteran insects with shared early developmental traits

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**Abstract** We discovered extremely small genomes (1C ~100 Mb) in the dipteran insects *Coboldia fuscipes* (Scatopsidae) and *Psychoda cinerea* (Psychodidae). The small genomes of these species cannot be explained by a fast developmental rate, which has been shown to correlate with small genome sizes in animals and plants but might accommodate the combined effects of other developmental traits, including small egg size, thin blastoderm layer, and long-germ development.

**Keywords** Diptera · Genome size · Egg size · Blastoderm · Pair-rule patterning · Flow cytometry

## Introduction

Genome size evolution is a complex multifactorial process. However, in animals and plants, genome size often correlates with cell size and the rate of cell division (Gregory 2001; Gregory 2002). The coding genome of animals seems to impose a lower threshold at about 100 Mb for the haploid (1C) genome; smaller genomes may require a substantive reduction of the coding genome (Lynch 2007). The 100-Mb threshold coincides for example with the genome size of *Caenorhabdites elegans* (Bennett et al. 2003), a very fast developing organism with about 19,000 protein-coding genes. The smallest insect genomes reported so far belong to highly specialized parasitic species including the louse *Pediculus humanus* (1C=105 Mb; Johnston et al. 2007) and the strepsipteran *Caenocholax fenyesi texensis* (1C=108 Mb; Johnston et al. 2004). The next to follow in size are the genomes of certain dipteran midges (*Mayetiola destructor*, Cecidomyidae; *Prodiamesa olivacea*, Chironomidae) with 1C>120 Mb (Zacharias 1979; Petitpierre 1996; Johnston et al. 2004), but genome size estimates of other dipterans are distinctly larger (1C> 140 Mb, Gregory et al. 2007).

A recent study surveying the genome sizes of 67 drosophilid species provided evidence for a significant positive correlation between genome size and developmental time, which suggests that, as a rule, species with smaller genome sizes develop more quickly than those with larger genomes (Gregory and Johnston 2008). Another developmental factor, egg size, might influence the genome size of dipteran insects as well. Dipterans establish a molecular prepattern of the segmented body in the blastoderm, the monolayer of cells surrounding the yolk before the onset of gastrulation (long-germ development, Davis and Patel 2002). Pattern formation up to this stage proceeds through a cascade of spatially regulated transcription factors in which pair-rule genes, the first genes to become activated in a reiterated pattern of transverse stripes, allocate cells of the blastoderm to alternate segments (Pankratz and Jäckle

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1993). The number of stripes of a particular pair-rule gene that is synchronously expressed in the cellular blastoderm varies in Diptera between six and eight, but its minimal spacing (measured in cell diameters) is conserved across the taxon (our unpublished data and Bullock et al. 2004; Lott et al. 2007; Lemke and Schmidt-Ott 2009). Based on the apparent spatial requirements for synchronous pair-rule patterning in the cellular blastoderm, one might expect that dipterans with small eggs package blastoderm cells more tightly than dipterans with large eggs. In extreme cases, tighter packaging of blastoderm cells could potentially correlate with particularly small genome sizes (small nuclei). To explore this idea, we examined the blastoderms and genome sizes of *Coboldia fuscipes* (Scatopsidae), *Psychoda cinerea* (Psychodidae), and *Chironomus riparius* (Chironomidae), three unrelated slowly developing species with unusually small eggs (<300 μm long, cf. Table 1 and Hinton 1981; Ferrar 1987), and compared these species to *Clogmia albipunctata* (Psychodidae), a close relative of *P. cinerea* with an egg length of ~400 μm and to *Drosophila melanogaster* (egg length, ~470–600 μm, Lott et al. 2007).

## Materials and methods

### Flow cytometry

Genome sizes of *C. fuscipes*, *P. cinerea*, *C. albipunctata*, and *C. riparius* were estimated using brain nuclei and a propidium iodide flow cytometry protocol (Bennett et al. 2003; DeSalle et al. 2005; Gregory and Johnston 2008). Briefly, sample and standard were ground together and passed through a 50-μm filter, stained with 50 ppm of propidium iodide and run (after 30 min in the cold and dark) in a Beckman Coulter Elite flow cytometer with the laser emitting 25 mW of exciting light at 488 nm. Fluorescent nuclei were detected using a high bandpass filter (615 nm). *P. cinerea* was prepared as above at the Jodrell Laboratory, RBG Kew, UK and run on a Partek CyFlow available at that laboratory (514-nm laser excitation, 25-mW, long pass filter). DNA content was determined by multiplying the ratio of the mean peak fluorescence of the 2C sample/mean peak of the standard with 175 Mb, the genome size of the sequenced strain of *D. melanogaster* anchored against the fully sequenced *C. elegans* strain (Bennett et al. 2003).

### Blastoderm measurements

To determine the number of transverse cell rows along the anteroposterior axis, late blastoderm embryos of *C. fuscipes*, *C. riparius*, *C. albipunctata*, and *D. melanogaster* were fixed with formaldehyde, stained with 4′,6-diamidino-2-phenylindole or Draq5, and examined with a confocal microscope. Internuclear distances were measured using ImageJ software. For *P. cinerea*, approximate internuclear distances were estimated by multiplying the length of eggs with an empirically determined correction factor (1.1, estimated from the other species) and dividing the product by the number of nuclear columns.

**Table 1** Egg length, blastoderm, and genome sizes

| Taxon | Egg-length | Nuclear Columns (AP-Axis) | Inter-Nuclear Distance Measurements | Haploid Genome Size (SE) | Sex | N |
|---|---|---|---|---|---|---|
| *Chironomus riparius (Chironomidae)* | | | | | | |
| Live (SE) | 258 μm (2.2) | 58.8 (1.3) | 4.87 μm (0.15) | 196.2 Mb (1.0) | F | 9 |
| Fixed (SE) | 258 μm (4.8) | | | 194.3 Mb (1.1) | M | 8 |
| *Coboldia fuscipes* (Scatopsidae) | | | | | | |
| Live (SE) | 252 μm (1.7) | 53.5 (0.6) | 4.65 μm (0.08) | 102.2 Mb (0.3) | F | 5 |
| Fixed (SE) | 229 μm (2.4) | | | 100.4 Mb (0.3) | M | 9 |
| *Psychoda cinerea* (Psychodidae) | | | | | | |
| Live (SE) | ~235 μm | 59.3 (0.7) | 4.19 μm (0.08) | 100.7 Mb (0.0) | n.d. | 3 |
| Fixed (SE) | 225 μm (5.9) | | | | | |
| *Clogmia albipunctata* (Psychodidae) | | | | | | |
| Live (SE) | 432 μm (2.3) | 75.0 (1.5) | 5.88 μm (0.10) | 316.6 Mb (1.4) | F | 3 |
| Fixed (SE) | 401 μm (6.0) | | | 316.6 Mb (0.5) | M | 2 |
| *Drosophila melanogaster* (Drosophilidae) | | | | | | |
| Live (SE) | 497 μm (3.6) | 84.4 (1.1) | 6.18 μm (0.11) | 175 Mb | F | – |
| Fixed (SE) | 462 μm (9.6) | | | | | |

Egg lengths of formaldehyde-fixed material was treated with commercial bleach, which removes the outer chorion

*F* female, *M* male, *n.d.* not determined, *SE* (numbers in brackets), standard error

## Results and discussion

To estimate the number and packaging density of blastoderm cells, we counted in each species the number of transverse rows of nuclei from the anterior to the posterior pole (Table 1). The number of rows in the cellular blastoderms of *C. fuscipes* (~54), *P. cinerea* (~59), and *C. riparius* (~60) was significantly lower than in *C. albipunctata* (~75) and *D. melanogaster* (~86). The average internuclear distances were ~4.7 µm in *C. fuscipes*, ~4.2 µm in *P. cinerea*, and ~4.4 µm in *C. riparius*, compared to ~5.9 µm in *C. albipunctata* and ~6.2 µm in *D. melanogaster*. Thus, the small size of *C. fuscipes*, *P. cinerea*, and *C. riparius* eggs correlates with both a reduction of the number of blastoderm cells and tighter packaging of these cells.

To estimate genome sizes, we used flow cytometry with *D. melanogaster* as standard (1C=175 Mb, Bennett et al. 2003). We estimated haploid genome sizes of ~100 Mb for *C. fuscipes* and *P. cinerea*, of ~200 Mb for *C. riparius*, and of more than 300 Mb for *C. albipunctata* (Fig. 1, Table 1). The small genome sizes of the cosmopolitan species *C. fuscipes* and *P. cinerea* seem to have evolved independently given that the two species are not closely related and that much larger genomes have been estimated for other psychodids, including *C. albipunctata* (this study), *Telmatoscopus meridionalis* (referenced in Gregory et al. 2007), *Phlebotomus papatasi* and *Lutzomyia longipalpis* (J. S. J., unpublished data). Furthermore, the small genome sizes of *C. fuscipes* and *P. cinerea* cannot be explained by an unusually fast developmental rate. At 25°C, the tiny eggs of *C. fuscipes* hatched as larvae after ~66 h (24 h in *D. melanogaster*), pupated after ~15 days (5 days in *D. melanogaster*), and emerged as adults after a total developmental time of ~19 days (see also Choi et al. 2000). Mating was observed on the following day and egg deposition 2 days after the adults had emerged from the pupal casing. The females deposited a single package of hundreds of eggs, which started development immediately after deposition. Thus, the total generation time of *C. fuscipes* was about 3 weeks (~12 days in *D. melanogaster*). In *P. cinerea*, embryogenesis lasted about 3 days (measured at 24°C). The eggs matured simultaneously (about 140–190, Sander 1985), and the entire life cycle lasted about 2–3 weeks. In summary, both species, like two other species measured in this study, *C. albipunctata* (embryogenesis, ~2 days; life cycle, 22 days at 25°C) and *C. riparius* (embryogenesis, ~2 days; life cycle, 3–4 weeks at 25°C), developed significantly slower than *D. melanogaster*.

While the genome size estimates of *C. fuscipes* and *P. cinerea* are consistent with our starting hypothesis that dipterans with extremely small eggs tend to have very small genomes, the twofold larger genome size of *C. riparius* (which shares a similar egg length and packaging density of

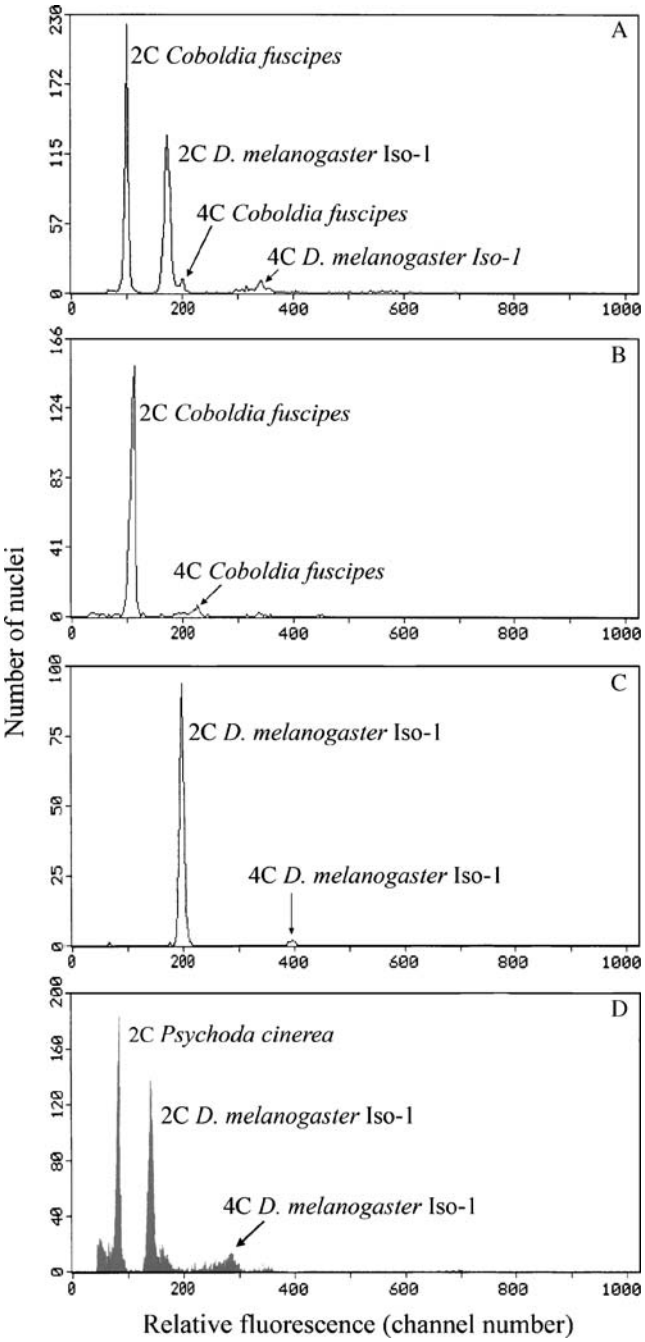


**Fig. 1** Sample histograms from the flow cytometric analysis. Diagrams show the number of nuclei with differing levels of red fluorescence corresponding to binding of propidium iodide to the DNA of 2C and 4C nuclei. **a** Co-prepared head cells of *C. fuscipes* and *D. melanogaster* Iso-1 (1C=175 Mb). **b** Head cells of female *C. fuscipes*. On a log/log scale, smaller peaks were not detected, indicating that the 2C peak of *C. fuscipes* is correctly identified. Ninety-six percent of the head cells were 2C and 4% 4C. We obtained reproducible results 30 min and 6 h after staining the cells. DNA degradation was not observed. **c** Head cells of female *D. melanogaster* Iso-1. **d** Co-prepared head cells of *P. cinerea* and *D. melanogaster* Iso-1

blastoderm cells) indicates that small egg size is not sufficient to explain the minute genomes of *C. fuscipes* and *P. cinerea*. To explain the differences in genome size between these species, we propose that upper thresholds also depend on the blastoderm type. Many lower dipterans (including *C. fuscipes*, *P. cinerea*, and *C. albipunctata*) develop a very thin blastoderm, in which the individual cells are essentially filled by the nucleus, while culicomorphan mosquitoes (including *C. riparius*) and cyclorrhaphan flies (including *D. melanogaster*) form a thickened blastoderm with a much smaller nucleus to cytoplasm ratio (Anderson 1972; Sander 2000; Bullock et al. 2004), which might attenuate constraints on the DNA content of blastoderm nuclei in species with small eggs.

In summary, we have identified two unrelated dipterans with extremely small genomes that share small egg size, thin blastoderm, and high packaging density of blastoderm cells. In both species, the advantages of synchronous pair-rule patterning (to reduce developmental time) and small egg size (to maximize offspring, in both species all eggs mature simultaneously and compete for space in the female abdomen) might have been traded against genome size. To put this new idea to a rigorous test, it will be necessary to search systematically for dipterans with tiny eggs, thin blastoderm, and slow development and to determine their genome sizes.

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